Bee Research Laboratory

Plant Sciences Institute

Brief Program Review
March 21, 1996

Agricultural Research Service

United States Department of Agriculture


United States
Department of
Agriculture
National Agricultural Library

TABLE OF CONTENTS

AGENDA
Brief Review - Bee Research Laboratory
Thursday, March 21, 1996
Conference Room 020, Bldg. 003

8:30	Opening Remarks	Dr. B. Leonhardt
8:35	Introductions	Dr. H. Shimanuki
8:40	Comments	Dr. J.van Schilfgaarde
8:45	BRL Overview	Dr. H. Shimanuki
9:00	Parasitic Mite Syndrome and the Control of Wax Moths	Dr. H. Shimanuki
9:15	Studies on the Biology of *Varroa jacobsoni*	Dr. W. A. Bruce
9:30	The Use of Natural Products for the Control of Parasitic Mites	Dr. N. W. Calderone
9:45	Control of Honey Bee Pests and Pathogens	Dr. M. F. Feldlaufer
10:00	Bee Diseases of Viral Origin	Dr. A. C. F. Hung
10:15	Break	
10:30	Molecular Systematics of *Apis* spp.	Dr. W. S. Sheppard* Dr. H. Shimanuki
10:45	Cryopreservation of Bee Germplasm and Sperm Identification	Dr. A. M. Collins
11:00	Biology and Management of Pollen Bees Important to Eastern Crops	Dr. S. W. T. Batra
11:15	Executive Session	Dr. B. Leonhardt

* Dr. Sheppard resigned Feb. 1996

MISSION STATEMENTS

Research, Education, and Economics

The mission of Research, Education, and Economics is to create, apply, and transfer knowledge and technology to provide affordable food and fiber, ensure food safety and nutrition, protect the environment, and support the rural development and natural resource needs of people by conducting integrated national and international research, information, education, economic, and statistical programs and services that are in the national interest.

Plant Sciences Institute

The Plant Sciences Institute research mission is to develop biological, chemical, and physical processes and principles including bioregulation that will improve pest management systems, improve crop production efficiency, improve conservation of natural resources, improve environmental quality, support regulatory and action agencies, and contribute to advances in biotechnology, and other societal benefits. The Institute's mission is accomplished through complex and exceptionally difficult fundamental and applied research programs in 16 laboratories.

Bee Research Laboratory

The Bee Research Laboratory (BRL) conducts research on the biology and control of honey bee diseases and parasites to ensure an adequate supply of bees for pollination and honey production. Specifically, scientists are conducting research on the biology and control of two parasitic mites, *Acarapis woodi* and *Varroa jacobsoni*, and chalkbrood disease. Additionally, scientists conduct research on the molecular systematics of Apis and on developing molecular methods for the identification of Africanized honey bees. The BRL also conducts research on the utilization of non-Apis bees for the pollination of crops of economic importance. Because of the research specialties, the BRL scientists provide authoritative identification of Africanized honey bees and the diagnosis of bee diseases and pests for Federal and State regulatory agencies and beekeepers on a worldwide basis.

INTRODUCTION

The Bee Research Laboratory (BRL), the oldest of the federal bee labs, is located on the USDA's Beltsville Agricultural Research Center. Federal honey bee research in the Washington metropolitan area had its beginning in 1891 and, except for a short break in 1896-1897, has been continuous for more than a century. The BRL conducts research on the biology and control of honey bee (*Apis mellifera* L.) diseases and parasites to ensure an adequate supply of bees for pollination and honey production. Specifically, scientists are conducting research on the biology and control of two parasitic mites, *Acarapis woodi* and *Varroa jacobsoni*, and American foulbrood and chalkbrood disease. Additionally, scientists conduct research on the molecular systematics of *Apis*, population genetic changes associated with colonization, and on developing molecular methods for the identification of Africanized honey bees. Recently, the BRL's research program was improved with the addition of Drs. Mark Feldlaufer and Anita Collins. Dr. Feldlaufer was added to provide biochemical expertise and Dr. Collins will be studying identification and preservation of bee germplasm. The BRL also conducts research on the utilization of non-*Apis* bees for the pollination of crops of economic importance. Because of the research specialties, BRL scientists provide authoritative identification of Africanized honey bees and diagnosis of bee diseases and pests for Federal and State regulatory agencies and beekeepers on a worldwide basis. The bee disease diagnostic service is probably the oldest, continuous service being offered by the USDA. This service was first offered in the late 1890's and has been continuous since then. Currently this service receives about 2,000 samples a year for disease and mite diagnosis.

RESEARCH ACCOMPLISHMENTS

Control of Honey Bee Diseases and Parasitic Mites

Problem addressed: The need to control two bacterial diseases of honey bees, *Bacillus larvae*, the causative agent of American Foulbrood and *Melissococcus pluton*, the agent of European Foulbrood).

Major findings: The 18-carbon fatty acid linoleic acid was isolated from the fungus *Ascosphaera apis,* and was shown to be an effective antimicrobial agent effective against both foulbrood bacteria. Other saturated and unsaturated free fatty acids were evaluated for their ability to inhibit bacterial growth, and lauric acid is currently being evaluated for its ability to prevent and control American and European foulbrood diseases of honey bees.

Significance: Currently, the only chemical approved by the Food and Drug Administration for foulbrood prevention and control is oxytetracycline. The impact of using fatty acids to prevent and control foulbrood disease is manifold, as these compounds would be safe, environmentally compatible, and cost-effective, which should appeal to 100% of the bee-keeping industry.

Problem addressed: At least 16 viruses have been found in honey bees, nine of them are known to occur in the U.S. Recently, the U.S. beekeeping industry has been beset with an unexplained high mortality of colonies, both adult bees and brood are affected and parasitic mites have been implicated. We named this condition, "bee parasitic mite syndrome" (BPMS). One possible cause is that inapparent virus (es) are activated by *Varroa jacobsoni.*

Major findings: Acute paralysis virus (APV) has been found as a cause of both adult bee and brood mortality in colonies in mainland Europe infested with the parasitic mite *V. jacobsoni.* We have developed biochemical and immunological techniques for the identification of APV and Kashmir bee virus (KBV). A strain of KBV was found in dead honey bees in the U.S. for the first time. The first case of both APV and KBV occurring in the same honey bee colony was also documented. However, there was no mixed infection of these two viruses in the same bee.

Significance: These results will facilitate our research program to determine the roles *Varroa* mites and viruses play so that strategies can be designed to help U.S. beekeepers and bee regulators diagnose and control BPMS.

Problem Addressed: To determine the presence or absence of the KBV in the United States because of virus' association with the parasitic mite, *Varroa jacobsoni*. To provide appropriate state and federal agencies with information relative to the importation of honey bees from countries where the virus was known to occur.

Major Findings: The following viruses were detected from at least one colony in each of ten apiaries in each of seven different states (CA, FL, ME, MN, NY, TX, WA): sacbrood virus, black queen cell virus, APV, cloudy wing virus, and KBV. These results were provided to the Apiary Inspector's office of each state and to APHIS.

Significance: The KBV was shown to be present in each of the seven states sampled and may be present in many or all remaining states. Appropriate state and federal agencies now have the information necessary to aid in decisions regarding the importation of honey bees from areas of the world where the viruses are known to occur.

Problem Addressed: What is the effect of water loss on the survival and behavior of *Varroa jacobsoni*?

Major Findings: The effect of water loss on the survival of small arthropods is extremely critical because of their large surface area to volume ratio. In general, water loss was greatest at high temperatures and low relative humidities for adult female mites during the spring and summer. However, the rate of 50% moisture loss was more rapid (2X) in winter across all humidities and temperatures tested except 97% RH and 34°C. In addition, mites in the winter weighed considerably less than those in spring and summer and therefore had less water to lose.

Significance: These results suggest that a fundamental difference exists between the water balance physiology of mites during summer and winter months when no brood is present. Because these mites evolved in the tropics, winter survival in temperate climates may represent a weak point in the parasitic mite's life cycle.

Molecular Genetics of Honey Bee Races and Populations in North America

Problem addressed: The need to identify germplasm diversity in US honey bee populations for a potential use in breeding bees resistant/tolerant to diseases and parasitic mites.

Major Findings: US feral honey bee populations exhibit significant levels of interpopulational genetic variation, as measured by mtDNA and allozymes. Genetic markers from various subspecies introduced into the US during the last century were found in the feral population. Commercial queen producing colonies exhibit reduced levels of genetic variability, although eastern and western queen producing regions represent largely different gene pools.

Significance: Bee breeding programs (and queen producers) can maximize the genetic diversity of their starting populations by including germplasm from the US feral population in their screening programs. Further, use of queens from both eastern and western queen producing regions, will help beekeepers maximize the diversity within their operations.

Problem addressed: The need to improve detectability of the Africanization process, especially following hybridization between EHB and AHB.

Major Findings: The use of a "composite" haplotype approach for mtDNA RFLP analysis permitted the discrimination of supra- and sub-Saharan honey bee subspecies. Genetic markers characteristic of an African honey bee subspecies introduced in the US in the 1860's were found in US feral populations. Approximately 25% of Argentine AHB populations have mtDNA of north African/Spanish origin. Microsatellite DNA variation was identified in a honey bee genomic library.

Significance: The composite haplotype approach can be used to more accurately detect the introgression of genes between EHB and AHB, thereby enabling regulators to better assess the consequences of Africanization in the US. Likewise, certain false "positives" for AHB, as detected by previous mtDNA methodology, are eliminated. Evidence for higher levels of gene flow between AHB and EHB populations, increases the probability that US beekeepers can have a significant positive influence in reducing the genetic effects of Africanization in feral honey bee populations.

Problem addressed: The need to develop a method for subspecies identification using honey bee sting remnants from victims. Because of the sensitivity of the public to Africanized honey bees, the media may blame many stinging incidences to Africanized honey bees even in areas where no Africanized honey bees are known to exist.

Major Findings: A mtDNA-based PCR method for identifying honey bee sting remnant has been developed. The technique enables us to remove a single sting from a victim and identify the racial origin of the honey bee.

Significance: This method could help allay fears of the public that Africanized honey bees are found in certain areas and help prevent beekeepers from being falsely accused of maintaining Africanized honey bee populations.

Problem addressed: We needed to establish the reliability of replacing the USDA Fast Africanized Bee Identification System with a mtDNA based method to identify suspect Africanized honey bee populations.

Major Findings: Approximately 6-7% of the morphometrically identified Africanized honey bee colonies sampled from Texas had European mtDNA.

Significance: This should serve as a warning to states that intend to screen for AHB using mtDNA. Such testing could misidentify 6-7% of the swarms as European when in fact the progeny are Africanized as determined by the official computer assisted morphometrics program (USDA-ID).

PRODUCTIVITY SUMMARY
(Since Last Review, Dec. 1992)

CATEGORY 1 SY's	Peer Reviewed Manuscripts		Senior Author Credit (Total)	Non-peer Reviewed			TOTAL
	FIRST AUTHORED	CO-AUTHORED		ABSTRACTS	POPULAR ARTICLES	SCIENTIFIC PRESENTATIONS	
SHIMANUKI	2	13	2	4	6	14	39
BATRA	9	3	9	2	3	10	27
BRUCE	5	1	5	2	-	9	17
CALDERONE	7	3	8	1	1	8	20
COLLINS	3	5	3	12	1	11	35
FELDLAUFER	5	4	6	1	-	6	19
HUNG	4	-	4	8	-	10	22
SHEPPARD*	4	14	10	4	-	8	30
TOTALS	39	49	47	34	11	76	209

* Resigned Feb. 1996

CRIS SUMMARY

IN-HOUSE CRIS PROJECTS

CRIS: 1275-21000-081-00D
Title: DIAGNOSIS AND CONTROL OF DISEASES OF HONEY BEES, INCLUDING PARASITIC BEE MITES
Net to Location: $973,018
Start: 06/25/93 Term: 06/24/98
SY: HACHIRO SHIMANUKI (0.80)
NICHOLAS W. CALDERONE (1.0)
WILLIAM A. BRUCE (1.0)
MARK F. FELDLAUFER (1.0)
AKEY C. HUNG (1.0)

CRIS: 1275-21220-025-00D
Title: MOLECULAR GENETICS OF HONEY BEE RACES AND POPULATIONS IN NORTH AMERICA
Net to Location: $225,367
Start: 03/28/94 Term: 03/27/99
SY: WALTER S. SHEPPARD (1.0)
HACHIRO SHIMANUKI (0.1)

CRIS: 1275-21220-022-00D
Title: CRYOPRESERVATION OF HONEY BEE SEMEN
Net to Location: $0; Balance Available Temporary Funding: FY 96, $143,924
Start: 07/02/95 Term: 09/30/96
SY: ANITA M. COLLINS (1.0)

CRIS: 1275-21000-073-00D
Title: UTILIZATION OF NON-APIS BEES FOR THE POLLINATION OF HORTICULTURE, SMALL FRUIT, AND VEGETABLE CROPS
Net to Location: $156,079
Start: 03/28/94 Term: 03/27/99
SY: SUZANNE W. T. BATRA (1.0)
HACHIRO SHIMANUKI (0.1)

USDA PILOT FUNDS

CRIS: 0500-00001-059-00D
Title: INFLUENCE OF QUEEN DEVELOPMENT TIME ON THE AFRICANIZATION OF EUROPEAN HONEY BEES
Net to Location: $0; Temporary Funding $11,693
Start: 10/01/93 Term: 09/30/96
SY: WALTER S. SHEPPARD (0.0)

OUTSIDE FUNDING

CRIS: 1275-21000-081-01R
Title: RESPONSE TO POLLINATION PROBLEMS FROM BEE MITES AND PATHOGENS, AND THE AFRICANIZED HONEY BEE
Net to Location: $0; Temporary Funding: $24,250 Total All FY's
Start: 01/01/94 Term: 12/31/96
SY: NICHOLAS W. CALDERONE (0.0)

CRIS: 1275-21000-081-03T
Title: FACTORS AFFECTING POLLINATION AND NECTAR COLLECTION BY THE HONEY BEE
Net to Location: $0; Temporary Funding: $40,000 Proposed Total All FY's
Start: 12/01/94 Term: 11/30/99
SY: NICHOLAS W. CALDERONE (0.0)

CRIS 1275-21000-081-04R
Title: INTEGRATED MANAGEMENT OF ACARAPIS WOODI AND VARROA JACOBSONI, MAJOR PESTS OF HONEY BEES
Net to Location: $0; Temporary Funding: $90,000 Proposed Total All FY's
Start: 09/01/95 Term: 08/31/98
SY: WILLIAM A. BRUCE (0.0)

Summary of Financial Resources

	FY '96	FY '97	FY '98
Net to Location	$1,495,038 1/	$1,339,421 2/	$1,339,421
Indirect Research Costs	306,831	338,136 3/	376,298
Adjustments 4/	30,000	30,000	30,000
Net to MU	1,188,207	1,001,285	963,123
Salary	965,715	832,300 5/	774,869
All-Other	222,492	169,678	188,254
Total Dollars per SY	185,492	141,868	160,303
Discretionary Funds per SY	23,187	20,267 6/	26,717
Percent Discretionary	12.41%	14.28%	11.97%
Percent in Salaries	65.26%	62.11%	57.85%

1/ Incl. $143,924 (temp. funding) for A. Collins
2/ Does not incl. Pilot funds ($11,693) and A. Collins funds ($143,924); but includes Sheppard
3/ Does not incl. A. Collins IRC $29,456; but includes Sheppard
4/ BARD, CRIS #1275-21000-081-04R, W. A. Bruce
5/ Does not incl. A. Collins salary ($78,679) and temp. positions; but includes Sheppard
6/ Excludes A. Collins; but includes Sheppard

MANAGERIAL CONCERNS

The BRL added two SYs since our last in-depth laboratory review in 1992. Dr. Mark Feldlaufer and his technician Kenneth Wilzer were transferred to the BRL on April 30, 1995. Dr. Anita Collins joined our staff on July 9, 1995. Dr. Steve Sheppard resigned from the BRL effective February 23, 1996. In addition, Rick Turcotte, Biological Science Technician resigned July 7, 1995. As a result of these changes, we now have approximately 7 FT support personnel to 7 SYs. We are in desperate need to recruit for a biological technician to help maintain the laboratory honey bee colonies. Dr. Batra does not have any technical help at the present time. Her CRIS is underfunded at $156,079 and unable to support technical help. We will temporarily assign David Vincent to work with Dr. Batra during the early Spring months prior to the honey bee season, this would meet her needs and would not interfere with David Vincent's assignment to Dr. Bruce.

Even though Dr. Sheppard, principal investigator (PI) resigned from the agency, CRIS 1275-21220-025-00D will be continued. Dr. Shimanuki will assume the role of PI until this CRIS is abolished at the end of FY96. Heui-Ra Yoo and Robin Wilcox will be assigned to Dr. Shimanuki to continue the Africanized honey bee identification service until they can be re-assigned to Dr. Collins.

The temporary CRIS 1275-21220-022-00D, Cryopreservation of Honey Bee Semen, will be abolished at the end of FY96 and the funds for will revert back to Headquarters. With the assistance and approval of the Plant Sciences Institute, the Beltsville Area and the National Program Staff, we intend to create a new CRIS which will include the identification of honey bee sperm using molecular methods, complete of studies on the molecular genetics of honey bee races in cooperation with Dr. Sheppard, Washington State University, and include the AHB identification service. Dr. Collins serving as the PI of this new CRIS which will include will include all the personnel from the two abolished CRIS's.

We have obtained four existing individual standing greenhouses from the Area for the support of the research on non-*Apis* bees. In addition we will refurbish our existing quarantine facility using R&M funds in FY96. We expect our backup generator for B-476 to be fully operational before the end of FY96. This will help ensure the preservation of irreplaceable frozen specimens required for the research on honey bee systematics.

SAFETY AND HEALTH REPORT

The Safety and Health Office inspected our facilities on March 1, 1996. There were no safety problems noted. The attic and storage areas are in the process of being reorganized.

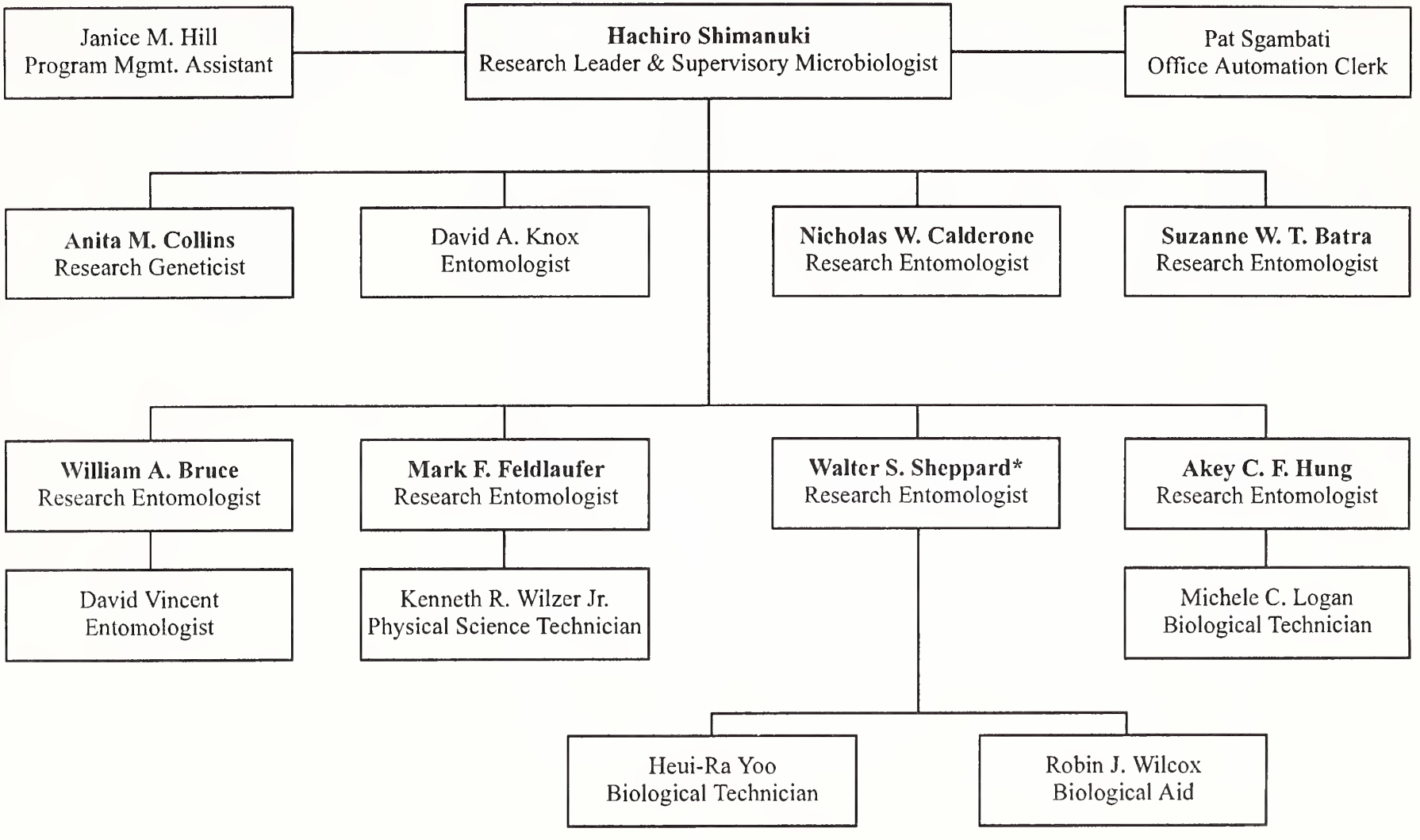
RESPONSE TO RECOMMENDATIONS FROM PREVIOUS LAB REVIEW

As a result of the last in-depth review held in December 1992, the following changes were implemented. Dr. Akey Hung was re-directed from developing a serologically-based AHB identification method to the study of honey bee viruses. The BRL also implemented the recommendation that Dr. Hung be sent to Rothamsted, England for a two-week training period to learn about bee viruses.

A second recommendation was to conduct a six-month evaluation on the progress being made by Dr. Bruce on developing an *in vitro* method for rearing *Varroa jacobsoni*. At the end of the six-month, the NPL and RL determined that this research was not progressing satisfactorily because of the lack of fundamental research on the biology of the mite and that this would reflect unfavorably on the productivity of Dr. Bruce. Consequently, Dr. Bruce was re-directed to work on the resistance and biology of parasitic mites.

Suggestions made by the in-depth review team regarding Dr. Batra's program were incorporated into a new CRIS which was established in March 1994 when the old CRIS expired. Another recommendation was that the BRL should provide greenhouse space for Dr. Batra. We believe we finally have the greenhouse space required by Dr. Batra. This is the third attempt to meet this need. The first attempt was to build a special greenhouse to Dr. Batra's specifications. However, the contractor defaulted and the greenhouse was never completed. Because we were unable to have a greenhouse built, in FY95 we requested an existing greenhouse from the Area. Since the requested greenhouse space in B-465 did not become available until March 1996 and the continued availability of the space in FY97 may be questionable, we requested four small freestanding greenhouses from the Area.

STAFFING CHART



* Resigned Feb., 1996

I. Name: Hachiro Shimanuki
Title: Research Leader & Supervisory Microbiologist

II. CRIS Project: 1275-21000-081-00D
CRIS Title: DIAGNOSIS AND CONTROL OF DISEASES OF HONEY BEES, INCLUDING PARASITIC BEE MITES

Progress: 1) An antibacterial substance was detected from the fungus, *Ascosphaera apis* which is the causative agent of honey bee chalkbrood disease. This substance was shown to be effective against American and European foulbrood diseases. Dr. Feldlaufer identified the active substance as the fatty acid linoleic acid. Using bioassays, subsequent studies identified another fatty acid, lauric acid as being more desirable from the standpoint of stability and efficacy. Field tests are underway to develop formulations for the use of lauric acid to control American foulbrood disease. **2)** A new condition of honey bees was described and named "Bee Parasitic Mite Syndrome" (BPMS). This condition is believed to be the result of a complex between a virus(es) and *Varroa jacobsoni*. **3)** CERTAN®, a *Bacillus thuringiensis* containing product for the control of the wax moth is no longer being manufactured. We are now screening candidate strains of *B. thuringiensis* in cooperation with a commercial firm for a new product to control the wax moth. **4)** After almost twenty years of preparation, the USDA Bee Bibliography has been adapted for computer retrieval. This bibliography which was started by the NAL in the late 1920's is the oldest bee bibliography in the English language. It was terminated with the advent of AGRICOLA in the early 1970's.
Plans: 1) We will continue field testing of lauric acid to formulate an efficacious mixture of lauric acid in an extender patty formulation. We also plan to evaluate the efficacy of this formulation in controlling the tracheal mite. **2)** We will continue work to identify symptoms, the interaction of virus and mite, and finally a control for BPMS. **3)** New strains of *B. thuringiensis* and formulations will be tested to control the wax moth. To increase beekeeper acceptance we will have to formulate the material to make it easy to apply and also to increase its longevity in the beehive. **4)** The work on the Bee Bibliography should be completed before the end of calendar year 1996.

III. Cooperators:

W. L. Biehn (IR-4)
Roger Morse (Cornell Univ.)
T. E. Rinderer (ARS, Baton Rouge)
Malcolm T. Sanford (Univ. of Florida)
Steve Sheppard (Washington State Univ.)
Skip Shieh (Environ. Associates)
Jack Thomas (Mann Lake Ltd.)
William Wilson (ARS, Weslaco)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Calderone, N. W. and Shimanuki, H. 1993. Distribution of tracheal mites among the mesothoracic tracheal trunks of the honey bee, *Apis mellifera*. Exp. Appl. Acarol. 17:663-672.

Feldlaufer, M. F., Lusby, W. R., Knox, D. A. and Shimanuki, H. 1993. Isolation and identification of linoleic acid as an antimicrobial agent from the chalkbrood fungus, *Ascosphaera apis*. Apidologie 24: 89-94

Feldlaufer, M. F., Knox, D. A., Lusby, W. R. and Shimanuki, H. 1993. Antimicrobial activity of fatty acids against *Bacillus larvae*, the causative agent of American foulbrood disease. Apidologie 24: 95-99.

Furgala, B., Duff, S. R. and Shimanuki, H. 1993. Evaluation of honey bee stocks from New Zealand. Amer. Bee J. 133:131-132.

Shimanuki, H. and Knox, D. A. 1993. Susceptibility of *Bacillus larvae* to Terramycin. Amer. Bee J. 134:125-126.

Calderone, N. W., Shimanuki, H. and Allen-Wardell, G. 1994. An in vitro evaluation of botanical compounds for the control of honeybee pathogens *Bacillus larvae* and *Ascosphaera apis*, and the secondary invader *B. alvei*. J. Essent. Oil Res. 6:279-287.

Schiff, N. M., Sheppard, W. S., Loper, G. R. and Shimanuki, H. 1994. Genetic diversity of feral honey bee (Hymenoptera: Apidae) populations in the southern United States. Ann. Entomol. Soc. Amer. 87:842-848.

Sheppard, W. S., Arias, M. C. and Shimanuki, H. 1994. Determination of mitochondrial DNA haplotypes from sting remnants of the honeybee *Apis mellifera* (Hymenoptera: Apidae). Bull. Entomol. Res. 84:551-554.

Shimanuki, H., Calderone, N. W. and Knox, D. A. 1994. Parasitic mite syndrome: The symptoms. Amer. Bee J. 134:827-828.

Bruce, W. A., Anderson, D. L., Calderone, N. W. and Shimanuki, H. 1995. A survey for Kashmir bee virus in honey bee colonies in the United States. Amer. Bee J. 135:352-355.

Calderone, N. W. and Shimanuki, H. 1995. Evaluation of four seed-derived oils as controls for *Acarapis woodi* (Acari: Tarsonemidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). J. Econ. Entomol. 88:805-809.

Hung, A. C. F., Adams, J. R. and Shimanuki, H. 1995. Bee parasitic mite syndrome (II): The role of Varroa mite and viruses. Amer. Bee J. 135:702-704.

Hung, A. C. F., Ball, B. V., Adams, J. R., Shimanuki, H. and Knox, D. A. 1996. A scientific note on the detection of American strains of acute paralysis virus and Kashmir bee virus in dead bees in one U. S. honey bee (*Apis mellifera* L.) colony. Apidologie: In Press.

Manuscripts Submitted for Publication

Feldlaufer, M. F., Calderone, N. A. and Shimanuki, H. Neutral sterol and ecdysteroid content of honey bee (*Apis mellifera*) drone pupae. Archives of Insect Biochemistry & Physiology

Sheppard, W. S., Shimanuki, H., Rinderer, T. E. and Garnery, L. Honey bees of the Americas. Nature

I. **Name: William A. Bruce**
Title: Research Entomologist

II. **CRIS Project: 1275-21000-081-00D**
CRIS Title: DIAGNOSIS AND CONTROL OF DISEASES OF HONEY BEES, INCLUDING PARASITIC BEE MITES

Progress: 1) All life stages of *Varroa jacobsoni* are being evaluated with regard to their water balance physiology and the effect on survival and behavior. Results indicate that the immobile deutonymph does not lose water at a rapid rate and is able to survive for many days without a great loss in body weight. 2) A scanning electron microscope (SEM) study is being conducted to determine if there is a structural or morphological basis that would support the hypothesis that the peritreme of *Varroa jacobsoni* may be involved in controlling water loss. Results indicate that this organ appears to open and close with changes in relative humidity. 3) A hypothetical model is being developed to help explain how the parasitic mite, *Varroa jacobsoni* is able to detect its host, *Apis mellifera*. It is proposed that the mite uses infrared (IR) electromagnetic radiation and that the receptors of this radiation are the thin-walled setae located at the tip of legs I. These structures have the structural characteristics and configuration necessary to function as dielectric antennas in the IR portion of the spectrum. The temperature of the honey bee is sufficient for the output of IR radiation at a radiant emittance that could be readily detected.
Plans: 1) Develop water rate-loss parameters of all life stages under various temperatures and relative humidities. 2) Subject adult females to specific temperatures and relative humidities and immediately examine the peritreme using SEM. 3) Refine the hypothetical model, continue setal measurements, and initiate observations of host/ parasite interaction.

CRIS Project: 1275-21000-081-04R
CRIS Title: INTEGRATED MANAGEMENT OF *ACARAPIS WOODI* AND *VARROA JACOBSONI*, MAJOR PESTS OF HONEY BEES

Progress: BARD - funded Sept. 1, 1995. Bee Research Laboratory: Automated, remote temperature and humidity sensors have been installed in honey bee colonies to determine the optimum time interval for data collection. Ohio State University: Tracheal mites are being counted from frozen bee samples to obtain a better understanding of the life cycle sequence of tracheal inhabitants over time. Hebrew University, Israel: Samples of honey bees, 20-30 hives/apiary from various parts of the country are being counted on a monthly basis to determine the level of infestation and the extent of damage.
Plans: Bee Research Laboratory: We will use temperature and humidity sensors and solar-controlled fans in colonies to determine the extent to which temperature and humidity can be changed. Ohio State University: Continue the sampling for tracheal mites. Hebrew University, Israel. Continue sampling for tracheal mites and determine extent of damage.

III. Cooperators:

U. Gerson (Hebrew University, Israel)
R. Mozes-Koch (Hebrew University, Israel)
A. Daq (Ministry of Agriculture, Israel)
C. Efrat (Ministry of Agriculture, Israel)
Y. Slabezki (Ministry of Agriculture, Israel)
Y. Stern (Ministry of Agriculture, Israel)
B. Yakobson (Kimron Veterinary Institute, Israel)
G. Needham (Ohio State University)
D. Sammataro (Ohio State University)
D. Wrensch (Ohio State University)
J. Kethley (Chicago Field Museum)
K. Hackett (IBL, BARC)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Bruce, W. A., Kethley, J. B. and Kaliszewski, M. J. 1993. Morphology of the gnathosoma of *Pyemotes tritici*: Chelicerae and an associated cheliceral structure (Acari: Pyemotidae). Int. J. Acarol. 29(2):1-10.

Bruce, W. A. and Kethley, J. B. 1993. Morphology of the gnathosoma of *Acarapis woodi*. (Acari: Tarsonemidae). Int. J. Acarol. 19(3):234-247.

Witherell, P. C. and Bruce, W. A. 1994. Control of *Varroa* mites on caged honey bees. Arthropod Management Tests 19:353.

Bruce, W. A., Anderson, D. L., Calderone, N. W. and Shimanuki, H. 1995. A survey for Kashmir bee virus in honey bee colonies in the United States. Amer. Bee Jour. 135(5):352-355.

Manuscripts Submitted for Publication

Bruce, W. A., Needham, G. R. and Potts W. J. E. Effects of temperature and water vapor activity on the survival of *Varroa jacobsoni* (Acari: Varroidae). Apidologie.

Bruce, W. A., Needham, G. R., Potts, W. J. E., and Vincent D. L. Water balance physiology and survival of *Varroa jacobsoni* removed from honey bee during winter (Acari: Varroidae). Expt. & Appl. Acarol.

I. Name: Nicholas W. Calderone
Title: Research Entomologist

II. CRIS Project: 1275-21000-081-00D
CRIS Title: DIAGNOSIS AND CONTROL OF DISEASES OF HONEY BEES, INCLUDING PARASITIC BEE MITES

CRIS Project: 1275-21000-081-01R
CRIS Title: RESPONSE TO POLLINATION PROBLEMS FROM BEE MITES AND PATHOGENS, AND THE AFRICANIZED HONEY BEE

Progress: 1) The evaluation of botanicals for control of parasitic mites has resulted in the description of seed-derived oils for control of the tracheal mite, *Acarapis woodi* and a blend of thymol and eucalyptus oil for control of *Varroa jacobsoni*. 2) We identified several factors affecting host-location by *Varroa*. These include larval sex, larval cell type, and age. We also found that mites discriminate among adult bees based on age and function. These findings suggest that both chemical and physical factors are involved in the host-location process. We have also developed a bioassay for evaluating mite response to volatiles and bee extracts.

Plans: 1) The use of sticky boards and drone combs as mite traps will be evaluated in depth. A reformulation of the thymol blend as a contact acaricide will also be evaluated. A second trial to evaluate the effects of botanicals on tracheal mites will be conducted.
2) We will attempt to identify specific compounds that are attractive, arrestant, or repellent to *Varroa*. We will also evaluate the effect of cell size on mite reproductive success and look for variation in attractiveness of larvae from different genetic backgrounds to *Varroa*.

CRIS Project: 1275-21000-081-03T
CRIS Title: FACTORS AFFECTING POLLINATION AND NECTAR COLLECTION BY THE HONEY BEE

Progress: I am presently investigating the use of larval extracts for increasing the pollination activity of honey bee colonies.
Plans: I plan to collect large quantities of cuticular extracts from honey bee larvae and determine whether or not this material is effective in altering the pollen collecting behavior of honey bee colonies.

III. Cooperators:

Malcolm T. Sanford (Univ. of Florida)
Marla Spivak (Univ. of MN)
Lois S. Willett (Cornell Univ.)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Calderone, N. W. 1993. Genotypic effects on the response of worker honey bees, *Apis mellifera*, to the colony environment. Anim. Behav. 46:403-404.

Calderone, N. W. and Shimanuki, H. 1993. Distribution of tracheal mites among the mesothoracic tracheal trunks of the honey bee, *Apis mellifera*. Exp. Appl. Acarol. 17:663-672.

Calderone, N. W. and Shimanuki, H. 1994. An *in vitro* evaluation of botanical compounds for the control of the honey-bee pathogens *Bacillus larvae*, *Ascosphaera apis*, and the secondary invader *Bacillus alvei*. J. Ess. Oil Res. 6: 279-287.

Shimanuki, H., Calderone, N. W. and Knox, D. 1994. Parasitic mite syndrome - I. The symptoms. Amer. Bee J. 134:827-828.

Bruce, W. A., Anderson, D. E., Calderone, N. W., and Shimanuki, H. 1995. Survey for Kashmir bee virus in honey bee colonies in the United States. Am. Bee J. 135: 352-355.

Calderone, N. W. 1995. Temporal division of labor in the honey bee, *Apis mellifera*: A developmental process or the result of environmental influences? Can. J. Zool. 73: 1410-1416.

Calderone, N. W. and Shimanuki, H. 1995. Evaluation of four seed-derived oils as controls for the tracheal mite, *Acarapis woodi* (Acari: Tarsonemidae), in colonies of the honey bee, Apis mellifera (Hymenoptera: Apidae). J. Econ. Entomol. 88: 805-809.

Calderone, N. W. and Spivak, M. 1995. Plant extracts for control of the parasitic mite, *Varroa jacobsoni* (Acari: Varroidae), in colonies of the honey bee, *Apis mellifera* (Hymenoptera: Apidae). J. Econ. Entomol. 88: 1211-1215.

Calderone, N. W. and Page, R. E. Jr. 1996. Temporal polytheism and behavioral canalization in the honey bee, *Apis mellifera*. Anim. Behav. In Press.

Manuscripts Submitted for Publication

Kuenen, L. P. S. and Calderone, N. W. Transfer of Varroa mites from newly-emerged bees: preferences for age- and function-specific adult bees. J. Exp. & Appl. Acarology

I. Name: Mark F. Feldlaufer (EOD May 1995)
Title: Research Entomologist

II. CRIS Project: 1275-21000-081-00D
CRIS Title: DIAGNOSIS AND CONTROL OF DISEASES OF HONEY BEES, INCLUDING PARASITIC BEE MITES

Progress: 1) Lauric acid is being evaluated in field colonies of honey bees for its ability to prevent and control foulbrood. Two formulations have been tried: (i) a dust consisting of confectioner's sugar coated with lauric acid, and (ii) a "grease" patty composed of a vegetable oil base and granulated sugar. Results from these tests need duplication, and a method that is relatively simple to implement developed. **2)** Sterol metabolism in the greater wax moth (*Galleria mellonella*) and in the parasitic mite *Varroa jacobsoni* is being investigated. Neutral sterol composition of used brood comb is being determined and compared to the tissue sterols of developing wax moth larvae. Different formulations of artificial diet are being used to rear *Galleria* on non-bee products, to facilitate studies with inhibitors of sterol metabolism. Sterols of *Varroa* have been compared to the sterols of their developing drone hosts, and the chemical nature of the molting hormone has been determined in both parasite and host.
Plans: 1) To develop a palatable, vegetable oil/sugar-based/lauric acid formulation that will prevent and control foulbrood diseases of honey bees under field conditions. **2)** To investigate how Δ24-reductase inhibitors of sterol metabolism can be incorporated into drawn comb so as to prevent damage by wax moth. Examine the fate of radiolabeled sterols injected into *Varroa* mites to determine how the parasitic mites handle the sterols from ingested honey bee hemolymph.

III. Cooperators:

Dr. Klaus Hartfelder (University of Tübingen, Tübingen, Germany)
Dr. Anna Rachinsky (University of Tübingen, Tübingen, Germany)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Feldlaufer, M. F., S. L. Buchmann, Lusby, W. R., Weirich, G. F. and Svoboda, J. A. 1993. The neutral sterols and ecdysteroids of the solitary cactus bee *Diadasia rinconis* Cockerell (Hymenoptera: Anthophoridae). Arch. Insect Biochem. Physiol. 23: 91-98.

Feldlaufer, M. F., Lusby, W. R., Knox, D. A. and Shimanuki, H. 1993. Isolation and identification of linoleic acid as an antimicrobial agent from the chalkbrood fungus, *Ascosphaera apis*. Apidologie 24: 89-94

Feldlaufer, M. F., Knox, D. A., Lusby, W. R. and Shimanuki, H. 1993. Antimicrobial activity of fatty acids against *Bacillus larvae*, the causative agent of American foulbrood disease. Apidologie 24: 95-99.

Lusby, W. R., Buchmann, S. L. and Feldlaufer, M. F. 1993. Pollen sterols from three species of sonoran cacti. Lipids 28: 469-470.

Weirich, G. F., Feldlaufer, M. F. and Svoboda, J. A. 1993. Ecdysone oxidase and 3-oxoecdysteroids reductases in *Manduca sexta* midgut: Developmental changes and tissue distribution. Arch. Insect Biochem. Physiol. 23: 199-211.

Svoboda, J. A., Feldlaufer, M. F. and Weirich, G. F. 1994. Evolutionary aspects of steroid utilization in insects. ACS Symposium Series 562. Isopentenoids and Other Natural Products. Evolution and Function. (W. David Nes, ed.). pp. 126-139.

Weirich, G. F., Kochansky, J. P., Masler, E. P., Lusby, W. R., Wagner, R. M., Feldlaufer, M. F. and Svoboda, J. A. 1994. Liquid scintillation counting of tritium-labeled neuropeptide in the subnanomolar range: Quantitative study of Adsorption to vials. Anal. Biochem. 216: 228-232.

Feldlaufer, M. F., Weirich, G. F., Imberski, R. B. and Svoboda, J. A. 1995. Ecdysteroid production in *Drosophila melanogaster* reared on defined diets. Insect Biochem. Molec. Biol. 25: 709-712.

Svoboda, J. A., Schiff, N. M. and Feldlaufer, M. F. 1995. Sterol composition of three species of sawflies (Hymenoptera: Symphyta) and their dietary plant material. Experientia 51: 150-152.

Schiff, N. M. and Feldlaufer, M. F. 1996. Neutral sterols of Symphyta and their relationship to other Hymenoptera. Lipids: In Press.

Manuscripts Submitted for Publication

Feldlaufer, M. F., Calderone, N. A. and Shimanuki, H. Neutral sterol and ecdysteroid content of honey bee (*Apis mellifera*) drone pupae. Archives of Insect Biochemistry & Physiology.

Weirich, G. F., William, V. and Feldlaufer, M. F. Ecdysone 20-hydroxylation in *Manduca sexta* midgut: Kinetic parameters of mitochondrial and microsomal ecdysone 20-monooxygenases. Archives of Insect Biochemistry & Physiology

I. Name: Akey C. Hung
Title: Research Entomologist

II. CRIS Project: 1275-21000-081-00D
CRIS Title: DIAGNOSIS AND CONTROL OF DISEASES OF HONEY BEES, INCLUDING PARASITIC BEE MITES

Progress: Initiated the honey bee virus research program in July, 1993. Developed antiserum for the identification of acute paralysis virus (APV) and Kashmir bee virus (KBV). Reported for the first time that a strain of KBV was found in dead honey bees in U. S. and that the two serologically related APV and KBV occurred in the same honey bee colony.
Plans: Develop protocols for extracting and sequencing RNA of APV and KBV. Develop RNA probes for the detection of picornaviruses in bee samples and their inapparent infections in honey bee tissues. Establish continuous cell cultures from *Apis mellifera*. Attempt to initiate an infection with the honey bee viruses in cell cultures. Develop new control strategies for parasitic mite syndrome in honey bees.

III. Cooperators:

J. R. Adams and D. Lynn (Insect Biocontrol Laboratory, BARC)
B. V. Ball (Rothamsted Experimental Station, UK)
C. Y. S. Peng (University of California, Davis)
C. Scott-Dupree (University of Guelph)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Hung, A. C. F. and Rubink, W. L. 1994. Tissue specificity and developmental expression of hexokinase and Africanized honey bee specific proteins in *Apis mellifera* L. (Hymenoptera: Apidae). Biochemical Systematics & Ecology, 22: 221-227.

Hung, A. C. F. and Wagner, R. M. 1994. Amino acid composition of an Africanized honey bee (Hymenoptera: Apidae) specific protein. Journal of Apicultural Research, 33: 113-117.

Hung, A. C. F., Adams, J. R. and Shimanuki, H. 1995. Bee parasitic mite syndrome (II): The role of *Varroa* mite and viruses. American Bee Journal 135: 702-704.

Hung, A. C. F., Ball, B. V., Adams, J. R., Shimanuki, H. and Knox, D. A. 1996. A scientific note on the detection of American strains of acute paralysis virus and Kashmir bee virus in dead bees in one U. S. honey bee (*Apis mellifera* L.) colony. Apidologie: In Press

I. Name: WALTER S. SHEPPARD (Resigned Feb. 1996)
Title: Research Entomologist

II. CRIS Project: 1275-21220-025-00D
CRIS Title: MOLECULAR GENETICS OF HONEY BEE RACES AND POPULATIONS IN NORTH AMERICA

CRIS Project: 0500-00001-059-00D
CRIS Title: INFLUENCE OF QUEEN DEVELOPMENT TIME ON THE AFRICANIZATION OF EUROPEAN HONEY BEES

Progress: **1)** Molecular and morphological investigations of feral honey bee colonies from the southern US indicates that this population is not a homogeneous mixture, but shows significant "structure", with genetic variation present among and within states. In addition, genetic markers from 19th century honey bee introductions were found in this population. **2)** Genetic variation in US commercial bee breeding populations is being investigated. Results indicate that these populations exhibit significantly less genetic diversity than feral and Old World populations. Preliminary studies of reciprocal crosses between Africanized honey bees (AHB) and European honey bees (EHB) indicate that queen development time of AHB is shorter than EHB, a possible mechanism for AHB spread and *Varroa* tolerance. **3)** Samples collected across an AHB/EHB transition zone in Argentina were reevaluated using a "composite haplotype" approach developed by BRL and baseline molecular markers from Old World honey bee subspecies. Results indicate that over 25% of the AHB collected from Argentina have mtDNA originating from Spanish/North African subspecies. Mitochondrial and morphological study of populations from regions of natural hybridization between *carnica/ligustica* (Italy - Austria) and *monticola/scutellata* (Kenya) demonstrate the existence of gene flow between endemic subspecies and the extent of genetic introgression. **4)** Continued investigation of subspecies genetic diversity has identified several methods that contribute to the discrimination of racial ancestry in New World populations. Methods are being developed to permit the use of ancient or archived DNA (e.g. from pinned specimens) and DNA extracted from honey bee stings for population genetic (mtDNA RFLP - Africanized honey bees) or systematic (DNA sequencing) studies.
Plans: **1)** Expand population genetic studies to encompass additional Old World honey bees (Africa, Russia), including subspecies known to have been introduced into the United States. **2)** Screen microsatellite primer pairs developed by BRL from honey bee genomic library for variability in populations and subspecies of the honey bee. Use microsatellites and other markers to investigate genetic interactions among feral, commercial and AHB populations in the US. **3)** Conduct preliminary analysis of genetic variation in *Varroa* populations using PCR-based methods already "on-line" in honey bee investigations.

III. Cooperators:

Drs. Niko and Gudrun Koeniger (Univ. of Frankfurt, Oberursel, Germany)
Marina Meixner (Univ. of Frankfurt, Oberursel, Germany)
Dr. Tom Rinderer (USDA-ARS)
Dr. Gloria Hoffman (USDA-ARS)
Dr. Bruce McPheron (Penn State University)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Meixner, M., Sheppard, W. S. and Poklukar, J. 1993. Asymmetrical distribution of a mitochondrial DNA polymorphism between two introgressing honey bee races. Apidologie 24:147-153.

Schiff, N. M. and Sheppard, W. S. 1993. Mitochondrial DNA evidence for the 19th century introduction of African honey bees into the United States. Experientia 49-530-532.

Rinderer, T. E., Oldroyd, B. P., Wongsiri, S., Sylvester, H. A., DeGuzman, L. I., Potichot, S., Sheppard, W. S. and Buchmann, S. S. 1993. Time of drone flight in four honey bee species in southeastern Thailand. J. Apic. Res. 32:27-33.

Rinderer, T. E., Sheppard, W. S. and Oldroyd, B. 1993. Africanized honey bees in the Americas. Scien. Amer. 269:84-90.

Last, J., Snider, R. H. Jr., Rosse, R. B., Deutsch, S. I., Sheppard, W. S., DeJong, D., Masters, C. F., Mefford, I. N. and Simpson, J. T. 1994. Preliminary evidence for differences in neurotransmitter concentrations in the brains of Africanized and European *Apis mellifera*. Rev. Bras. Genet. 17:149-151.

McPheron, B. A, Gasparich, G. E., Han, H. Y., Steck, G. J. and Sheppard, W. S. 1994. Mitochondrial DNA restriction map for the Mediterranean Fruit Fly, *Ceratitis capitata*. Biochem. Genet. 32:25-30.

Meixner, M. D., Krell, R., Dietz, A., and Sheppard, W. S. 1994. Morphological and Allozyme variability in honey bees from Kenya. Apidologie 25:188-202.

Schiff, N. M., Sheppard, W. S., Loper, G. R. and Shimanuki, H. 1994. Genetic diversity of feral honey bee (Hymenoptera: Apidae) populations in the southern United States. Ann. Entomol. Soc. Amer. 87:842-848.

Sheppard, W. S., Arias, M. C. and Shimanuki, H. 1994. Determination of honey bee mitochondrial DNA haplotypes from sting remnants. Bull. Entomol. Res. 84:551-554.

Gasparich, G. E., Sheppard, W. S., Han, H. Y., McPheron, B. A. and Steck, G. J. 1995. Analysis of mitochondrial DNA and development of PCR-based diagnostic molecular markers for Mediterranean fruit fly (*Ceratitis capitata*) populations. Insect Molec. Biol. 4:61-67.

Schiff, N. M. and Sheppard, W.S. 1995. Genetic analysis of commercial honey bees (Hymenoptera: Apidae) from the southern United States. J. Econ. Entomol. 88: 1216-1220.

Arias, M. C. and Sheppard, W. S. 1996. Molecular phylogenetics of honey bee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequences. Mol. Phylogen. Evol. In Press.

Azeredo-Espin, A.M.L., Schroder, R.F.W., Roderick, G.K. and W.S. Sheppard. 1996. Intraspecific mitochondrial DNA variation in the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Biochem. Genet. In Press.

Gasparich, G. E., Silva, J. G., Han, H. Y., McPheron, B. A., Steck, G. J. and Sheppard, W. S. 1995. Population genetic structure of Mediterranean fruit fly (Diptera:Tephritidae) and implications for worldwide colonization patterns. Ann. Entomol Soc. Amer. In Press.

Schiff, N. M. and Sheppard, W.S. 1996. Genetic differentiation in the queen breeding population of the western United States. Apidologie In Press.

Sheppard, W.S. and Arias, M.C. 1996. Biodiversity of the honey bee: value and preservation. **In** Proc. of the XXXIVth International Apicultural Congress (Apimondia), Lausanne, Switzerland, August, 1995. In Press

Sheppard, W. S., Rinderer, T. E., Melxner, M. D., Yoo, H. R., Stelzer, J. A., Schiff, N. M., Kamel, S. M. and Krell, R. 1996. *Hin*FI variation in mitochondrial DNA of Old World honey bee races. J. Hered. In Press.

Manuscripts Submitted for Publication

Sheppard, W. S., Shimanuki, H., Rinderer, T. E. and Garnery, L. Honey bees of the Americas. Nature:

I. **Name: Anita M. Collins** (EOD July 1995)
Title: Research Geneticist (Insects)

II. **CRIS Project: 1275-21220-022-00D**
CRIS Title: CRYOPRESERVATION OF HONEY BEE SEMEN

Progress: Identification of sperm from individual drones using mitochondrial DNA (mtDNA) analysis with subspecies markers, especially for Africanized bees, has been demonstrated. Also, collection of semen from spermathecae of mated queens and subsequent identification has been done.
Plans: Survey, using mtDNA evaluation, existing samples from south Texas/northeast Mexico for presence of subspecies markers in those samples already identified by morphometrics as Africanized. Research the usefulness of various diluents for the cryopreservation of honey bee semen based on recent advances in other species. Expand the usable markers for semen identification/certification to include nuclear polymoprhisms, especially those that might be relevant to Africanized genotypes. Evaluate the possibility of doing DNA analysis on eggs. Examine the possibility of cryopreservation of honey bee eggs or very young zygotes, as has been accomplished with *Drosophila*. Based on the results of the previous plans, develop a germplasm collection protocol for the honey bee.

III. Cooperators:

Gloria DeGrandi-Hoffman (Tucson, AZ)
William Rubink (Weslaco, TX)
Steve Sheppard (Washington State University)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Collins, A. M., Rubink, W. L., Cuadriello Aguilar, J. I. and Hellmich II, R. L. 1993. El uso de repelentes de insectos, contra abejas que defienden el nido. Apicultura Moderna. Mayo 6:27-32. In Spanish.

Villa, J., Rinderer, T. E. and Collins, A. M. 1993. Overwintering of Africanized, European and hybrid honey bees in the Andes of Venezuela. Environ. Ent. Physiol. & Chem. Ecol. 22:183-189.

Wilson, W. T., Baxter, J. R., Collins, A. M., Cox, R. L. and Cardoso-T., D. 1993. Formic acid fumigation for control of tracheal mites in honey bee colonies. Bee Science 3(1):26-32.

Collins, A. M., Daly, H., Rinderer, T. E., Harbo, J. R. and Hoelmer, K. 1994. Correlations between morphology and colony defense in *Apis mellifera* L. J. Apic. Research. 33(1):3-10.

Danka, R. G., Loper, G. M., Villa, J. D., Williams, J. L., Sugden, E. A., Collins, A. M. and Rinderer, T. E. 1994. Abating feral Africanized honeybees to enhance mating control of European queens. Apidologie. 25:520-529.

Eischen, F. A., Underwood, B. A. and Collins, A. M. 1994. The effect of delaying pollination on cantaloupes. J. Apic. Res. 33(3):180-184.

Sugden, E. A., Collins, A. M., Rubink, W. L., Bradley, L., Davis, K. and Quintero, E. 1994. Two years of "Africanization": honey bee stinging incidents in Texas. Bee Science. 3(2):68-75.

Pettis, J. S., Winston, M. L. and Collins, A. M. 1995. Suppression of queen rearing in European and Aricanized honey bees *Apis mellifera* L. by synthetic queen mandibular gland pheromone. Insectes Sociaux. 42:113-121.

Collins, W. M., Rubink, W. L., Cuadriello-Aguilar, J. I. and Hellmich, R. L. 1996. Use of insect repellents for dispersing defending honey bees (Hymenoptera: Apidae). J. Econ. Entomol. In Press

Rubink, W. L., Luevano-Martinez, P., Sugden, E. A., Wilson, W. T., and Collins, A. M. 1996. Subtropical *Apis mellifera* (Hymenoptera: Apidae) swarming dynamics and Africanization rates in northeastern Mexico and southern Texas. Annals Entomol. Soc. Amer. In Press.

Manuscripts Submitted for Publication

Williams, J.L., Rinderer, T.E. and Collins, A.M. Management of nuisance honey bees on federal lands. ARS publication.

I. **Name:** Suzanne W. T. Batra
Title: Research Entomologist

II. **CRIS Project:** 1275-21000-073-00D
CRIS Title: UTILIZATION OF NON-APIS BEES FOR THE POLLINATION OF HORTICULTURE, SMALL FRUIT, AND VEGETABLE CROPS

Progress: 1) Developed management technology and tested host range, effectiveness, phenology, and climatic tolerance of *Anthophora pilipes villosula* Sm., a gentle Japanese solitary bee that was introduced to pollinate apples, peaches, and blueberries in humid parts of USDA Plant Hardiness Zones 7-9, where honey bees are and will be under stress from parasites and Africanization. 2) Investigated the many species of bees (Apoidea) that pollinate apples and other orchard fruit crops at high elevations in the cold, remote, previously unsurveyed Great Himalaya Range of Garhwal, India; among the fruit pollinating bees was the world's largest honey bee, *Apis laboriosa* Sm., discovered 600 Km. west of its previously - known range. Many of the bees that were collected are expected to be new to science (new species); they were found near the center-of-origin of several of our temperate orchard crops, thus, they may be co-evolved to pollinate them efficiently. 3) Compared native North American pollinating bees in two regions: a temperate, broadleaf forest in the West Virginia Appalachian mountains, and a boreal forest in the New York Adirondack mountains (a U.N. Biosphere Reserve). The effect of diflubenzuron insecticide on bee populations; the exclusive pollination of potato flowers by, and use of honeydew by, bumble bees; and nematodes that are associated with native bees were investigated. As honey bee populations and forests decrease, it becomes more important to evaluate the biodiversity and roles of our native pollinators in forests and other ecosystems.
Plans: 1) To field-test *Anthophora pilipes villosula* Sm. on fruit crops in several areas in zones 7 - 9, in cooperation with scientists, growers, and selected beekeepers. 2) To identify or describe new species of bees from the Himalaya, and work with Indian scientists to survey the bees in more Himalayan areas; species are expected to be diverse and many endemic, due to geographical isolation. 3) To complete research on the ecology and behavior of native bees of northeastern forests, and to attempt the practical management of additional species of solitary bees for pollination of fruit crops (for example, *Anthophora abrupta*, *Colletes* spp.).

III. Cooperators:

E. M. Barrows (Georgetown University)
H. M. Fales (National Institutes of Health)
R. D. Gaur (Garhwal University, India)
R. M. Giblin-Davis (University of Florida)
K. V. Krombein (Smithsonian Institution)
Y. Maeta (Shimane University, Japan)
B. Melching (Cornell University)
B. B. Norden (Smithsonian Institution)
S. F. Sakagami (Hokkaido, Japan)

IV. Publications (since last review, Dec. 1992):

Peer Reviewed

Batra, S. W. T. 1993. Opportunistic bumblebees congregate to feed at rare, distant, alpine honeydew bonanzas. J. Kansas Entomol. Soc. 66(1):125-127.

Batra, S. W. T. 1993. India's buzzy biodiversity of bees. Current Science 65 (3):377-280.

Batra, S. W. T., Sakagami, S. F., and Maeta, Y. 1993. Behavior of the Indian allodapine bee *Braunsapis kaliago*, a social parasite in the nests of *B. mixta* (Hymenoptera: Anthophoridae). J. Kansas Entomol. Soc. 66 (3):345-360.

Batra, S. W. T. 1993. Male-fertile potato flowers are selectively buzz-pollinated only by *Bombus terricola* Kirby in upstate New York. J. Kansas Entomol. Soc. 66 (2):252-254.

Giblin-Davis, R. M., Mundo-Campo, M., Baldwin, J. G., Norden, B. B. and Batra, S. W. T. 1993. Description of *Bursaphelenchus abruptus* n. sp., Nemata, Aphelenchoidae, an associate of a digger bee. J. Nematol. 25 (2):161-172.

Batra, S. W. T. 1994. *Anthophora pilipes villosula* Sm. (Hymenoptera: Anthophoridae), a manageable Japanese bee that visits blueberries and apples during cool, rainy, spring weather. Proc. Entomol. Soc. Wash. 96 (1):98-119.

Norden, B. B., Krombein, K. V. and Batra, S. W. T. 1994. Nesting biology of *Exomalopsis (Phanamalopsis) solani* Cockerell (Hymenoptera: Anthophoridae). Proc. Entomol. Soc. Wash. 96:350-356.

Batra. S. W. T. 1995. The evolution of "eusocial" and the origin of " pollen bees". The Maryland Naturalist 39 (1-2):1-4.

Batra, S. W. T. 1995. Bees and pollination in our changing environment. Apidologie 26:361-370.

Batra, S. W. T. and Norden, B. B. 1996. Fatty food for their brood: how *Anthophora* bees make and provision their cells. Proc. Entomol. Soc. Washington In Press.

Manuscripts Submitted for Publication

Barrows, E. M. and Batra, S. W. T. Bumble bees (Hymenoptera: Apidae) in a central Appalachian broadleaf forest: species richness and abundances, flight periods and locations, and Diflubenzuron. J. Econ. Entomol.

Batra, S. W. T. Biology of *Apis laboriosa* Smith, a pollinator of apples at high altitude in the Greater Himalaya of Garhwal, India (Hymenoptera: Apidae). J. Kansas Entomol. Soc.